Metals Bioaccumulation Mechanism in Neem Bark

Kishore K. Krishnani, Veera M. Boddu, Deok Hyun Moon, S. V. Ghadge, Biplab Sarkar, M. P. Brahmane, K. Choudhary, V. Kathiravan, et al.

≋ Springer

Springer

CrossMark

Metals Bioaccumulation Mechanism in Neem Bark

Kishore K. Krishnani[1] · Veera M. Boddu[2] · Deok Hyun Moon[3] · S. V. Ghadge[1] ·
Biplab Sarkar[1] · M. P. Brahmane[1] · K. Choudhary[4] · V. Kathiravan[5] ·
Xiaoguang Meng[6]

Abstract The aim of this work was to define the bioaccumulation mechanism of metals onto the non-living biomaterial prepared from an extensively available plant bark biomass of neem (*Azadirachta indica*). Based on maximum ultimate fixation capacities (mmol/g) of the product, metals ions could be arranged as $Hg^{2+} < Cd^{2+} < Pb^{2+} \cong Cu^{2+}$. Surface properties of the biomaterial were characterized by X-ray photoelectron spectroscopy and X-ray diffraction techniques for their sorption mechanism. Whewellite ($C_2CaO_4 \cdot H_2O$) was identified in the biomaterial, which indicated that calcium ions are electrovalently bonded with carboxylate ions facilitating the ion exchange mechanism with metal ions. Bioaccumulation of metal ions was also studied by Fourier transform infrared spectroscopy, which indicated the presence of functional groups implicated in adsorbing metal ions. Biomaterial did not adsorb anionic As(III), As(V) and Cr(VI), because of their electrostatic repulsion with carboxylic functional groups. Neem bark can be used as bioindicators, bioaccumulators and biomonitors while determining environmental pressures. Metal bioaccumulative properties and structural investigation of plant bark has potential in providing quantitative information on the metal contamination in the surrounding environment.

Keywords Biomaterial · Characterization · Toxicants · Removal · Whewellite · Ion exchange

During recent years, interests have been primarily focused on metals due to their known toxicity, as they are discharged in small quantities into the environment by numerous activities. Human food chain effects have been of great concern, because of an increasing metal accumulation and their potential adverse health impact. For these reasons, legislations governing levels of contaminants are becoming more stringent. Unlike organic compounds, metals are non-biodegradable and tend to accumulate in plants (Bulut and Baysal 2006; Krishnani and Ayyappan 2006), which can be used as bioindicators, bioaccumulators and biomonitors while determining environmental pressures (Samecka-Cymerman et al. 2006). In biosorption, complexes are formed between metal ions and the ligands or functional groups of biological material. Mechanisms of metal uptake are not always known, especially in the plant barks, which are metal bioaccumulator. The examination of the functional groups is crucial in bioaccumulation of metals in the native or modified bark materials, but little attention has been paid to the identification of the active binding sites and cations involved in metal uptake (Gloaguen and Morvan 1997; Athar et al. 2007; Arshad et al. 2008; Maharia et al. 2010). In the present investigation, new biomaterial prepared from plant bark of neem (*Azadirachta indica*) has been evaluated for its bioaccumulation mechanism of Hg, Cd, Pb, Cu, As and Cr with respect to individual and multi component systems.

✉ Kishore K. Krishnani
krishnanik@hotmail.com

1 National Institute of Abiotic Stress Management, Baramati, Pune 413115, India

2 US Army Construction Engineering Research Laboratories, Champaign, IL, USA

3 Department of Environmental Engineering, Chosun University, Gwangju 501759, Korea

4 Directorate of Weed Science Research, Jabalpur, India

5 The Madura College, Madurai, TN, India

6 CEE, Stevens Institute of Technology, Hoboken, NJ 07030, USA

Materials and Methods

Metal stock solutions (10,000 mg/L) were prepared by dissolving appropriate amount (1 g) of metal salt [$CuCl_2 \cdot 2H_2O$, $HgCl_2$, $CdCl_2 \cdot 2.5H_2O$, $Pb(NO_3)_2$, $K_2Cr_2O_7$, $NaAsO_2$, NaH_2AsO_4] in 100 mL of deionized water.

Native plant bark material was subjected to 1.5 % alkali treatment (5:1 ratio of waste and NaOH) and then autoclaved for 20 min in order to remove the low molecular weight lignin compounds. This material was then washed with 0.001 N HCl until the pH of washing reached a constant 6.0 ± 0.1. This fraction was then dried in an oven at 50°C, ground to powder, and sieved at 400 μm.

Equilibrium batch experiments were conducted with stoppered flasks in a mechanical shaker incubated at $30 \pm 0.2°C$ to evaluate the biomaterial (2 g/L) for the removal of different metal ions separately at pH 6.0 ± 0.1. Prior to the introduction of metal ions, flasks were vigorously stirred for 1.5 h to hydrate the biomaterial. After the introduction of the metal (200 mg/L), pH was either naturally buffered or adjusted with 0.1 M HNO_3 to 6.0 ± 0.1. Metal solution was allowed to equilibrate with the biomaterial for 24 h in an incubator agitated at 200 rpm. After equilibration, samples were filtered through a 0.45 μm membrane filter and analyzed along with a control sample for metal ions and other cations such as Ca^{2+} and Mg^{2+} using Inductively Coupled Plasma (ICP) emission spectrometer (Varian Liberty-200, Palo Alto, CA). The amount of metal adsorbed (mg) per unit mass of biomaterial (Qe) was obtained as described by Krishnani et al. (2008a).

Two grams of biomaterial prepared from neem bark was packed into a column with an internal diameter of 1 cm and a column height of 15 cm. Column ends were fitted with filter disks (Millipore) of 0.45 μm pore size to retain the material. Aqueous solution containing 20 mg/L of four different metals (Pb, Cd, Hg, Cu) was passed through the column containing biomaterial at a flow rate of 1 mL/min at the predetermined pH. The bed volume of the column was 15 mL. Concentrations of metal ions and other cations such as Ca^{2+} and Mg^{2+} in column effluents obtained at various intervals were monitored using an ICP spectrometer (Varian-Liberty 200). The column runs were carried out to the point where the breakthrough capacity for most of the metal ions was consumed.

X-ray photoelectron spectroscopy and Fourier transform infrared spectra of the biomaterial after exposure to the metal ion solution were obtained on a PHI model 5400 axis Ultra Kratos Analytical instrument (Manchester, UK) and a Fourier transform infrared spectrometer-FT/IR 4100 in the range of 500–4000 cm^{-1} (Jasco Corp. Tokyo, Japan) respectively at US Army Engineer Research and Development Centre—Construction Engineering Research

Laboratory using the process as described by Krishnani et al. (2008a). Biomaterial was ground using a ceramic mortar and pestle in order to achieve a homogeneous and uniform sample. Step-scanned X-ray diffraction (XRD) patterns were collected by the Rigaku DXR-3000 computer-automated diffractometer. XRD analyses were conducted at 40 kV and 40 mA using Cu radiation with a diffracted beam graphite-monochromator. The XRD pattern was collected from 5° to 65° in 2θ with a step size of 0.02° and a count time of 3 s per step. Qualitative XRD analyses were performed using the JS Advanced Data Exchange (JADE) program after background removal. Qualitative analyses of XRD patterns were performed using Jade software version 7.1 (MDI 2005) and the International Center for Diffraction Data (ICDD) database (ICDD 2002).

Results and Discussion

The XPS survey spectrum of neem based lignocellulosic biomaterial loaded with metals identified two major elements (carbon and oxygen) in neem biomaterial. XRD pattern of biomaterial prepared from neem revealed low carbon contents, indicating the presence of inorganic phases (SiO_2, Fe_2O_3) in neem biomaterial. Whewellite was identified in the sample tested. Whewellite is a hydrated calcium oxalate mineral ($CaC_2O_4 \cdot H_2O$). Calcium ions are electrovalently bonded with carboxylate ions, which facilitate the ion exchange mechanism with metal ions.

The results of the maximum adsorption capacity of the neem biomaterial at $30 \pm 0.2°C$ and pH 6.0 ± 0.1 vis-à-vis maximum values adsorbed per unit mass of sorbents from other lignocellulosic materials reported in the literature are given in Table 1. Results are either comparable or considerably greater than those reported in the literature for other sorbents.

Neem biomaterial had a higher sorption capacity (mmol/g) for copper and lead, followed by cadmium and mercury (Table 2). Retention of metal ions onto the biomaterial increased in the order: $Hg^{2+} < Cd^{2+} < Pb^{2+} \cong Cu^{2+}$. The ratio Ca^{2+} and Mg^{2+} released/metal fixed expressing the percentage of ion exchange (or adsorption) indicated a comparable quantity of ions were released from the material, confirming the ion-exchange mechanism of metal species Cu^{2+}, Pb^{2+} and Cd^{2+}. This indicated the bark material contained enough Ca^{2+} ions to exchange with metals ions. In the case of Hg^{2+}, the release of Ca^{2+} and Mg^{2+} ions were not comparable with the maximum quantity adsorbed onto the substrate. It could be due to other cations involved in the ion exchange. Biomaterial did not adsorb anionic As(III), As(V) and Cr(VI), because of

Table 1 Maximum adsorption capacities (mg/g) for neem biomaterial in the present study (pH 6 ± 0.1) and other lignocellulosic materials in the literature

Material	Literature	Hg	Cd	Pb	Zn	Cu	Ni	Co	Mn
Lignocellulosic agrowastes									
Tannin adsorbent	Xin-Min et al. (2001)			30–40					
Adsorbent from condensed tannins	Xin-Min and Zhao (2003)			57.5–114.9					
Wheat bran	Bulut and Baysal (2006)			69–87					
Bagasse	Krishnani et al. (2009)	14	3.36	24.22	3.27	7.6	2.64	2.5	0.97
Rice husk	Krishnani et al. (2008a)	33.1	14.4	54	7.47	10.8	5.4	8.5	7.7
Paddy straw	Krishnani et al. (2008b)	43.75	14.9	58.8	12.6	15.6	9.24	9.5	9
Bark materials									
Black Oak	Masri et al. (1974)	400	25.9	153.3	–	–	–	–	–
Hardwood bark	Jang et al. (2005)			72.52	12.23	22.81	–	–	–
Manihotsculenta Cranz	Horsfall et al. (2006)		5.88–26.3		22.2–83.3	33.3–90.9	–	–	–
Hemidesmum indicus	Sekhar et al. (2004)	–	–	93.6	–	–	–	–	–
Azadirachta indica	Present study	18.9	27	66.46	–	24.1	–	–	–

Table 2 Metal ions sorption and corresponding ion exchange onto the biomaterial at pH 6.0 ± 0.1 (initial metal concentration 200 mg/L)

Metal	Metal ion fixed		Exchangeable cations					
	mg/g	mmol/g	Ca^{2+}		Mg^{2+}		Total	
			mg/g	mmol/g	mg/g	mmol/g	mmol/g	%
Pb	66.46	0.32	11.4	0.284	0.766	0.0314	0.315	99
Cu	24.1	0.378	13.9	0.3468	0.65	0.0268	0.374	99
Hg	18.9	0.0942	1.39	0.0347	0.427	0.0176	0.0523	55
Cd	27.0	0.24	8.70	0.217	0.366	0.015	0.232	97

their electrostatic repulsion with carboxylic functional groups.

Hardwood bark was a better adsorbent of metals in urban runoff compared with cypress barkpine bark nugget (Jang et al. 2005). *Hemidesmus indicus* was used as biomaterial to remove Pb from aqueous streams by batch and column experiments (Sekhar et al. 2004). Many factors such as ionic charge, ionic radii and electrode potential are involved in the complex reaction of metal removal capacity of the biomass. The greater the electrode potential, the greater the affinity for biomass (Sekhar et al. 2004).

Horsfall et al. (2006) investigated the kinetics of Cd^{2+}, Cu^{2+} and Zn^{2+} adsorption onto pure and thioglycolic acid treated cassava tuber bark wastes (CTBW), and the monolayer sorption capacity was found to range between 5.88–26.3, 33.3–90.9 and 22.2–83.3 mg/g, respectively for Cd^{2+}, Cu^{2+} and Zn^{2+}. Samecka-Cymerman et al. (2006) investigated the suitability of bark and pine needles to monitor metal pollution, and the study concluded that bark was a better accumulator of Cd, Cu, Ni and an inferior accumulator of Mn.

Experimental breakthrough curves for metal ion adsorption onto the biomaterial at 30 ± 0.2°C are shown in Fig. 1. Different flow rates were used to achieve the highest removal of the adsorbent which was 0.5 mL/min. There was no leakage of Pb^{2+} and Cu^{2+} throughout the bed volumes with an initial concentration (C_i) of 20 mg/L. For Hg^{2+} and Cd^{2+}, there was no leakage up to 30–35 bed volumes with an initial concentration (C_i) of 20 mg/L. After these bed volumes, the column effluent concentration (C_o) increased gradually and attained the influent concentration at around 60 bed volumes.

The fast rise of effluent to influent concentration with bed volumes indicated faster kinetics adsorption. The biomaterial exhibited greater adsorption capacity due to a higher initial concentration and optimum pH. The column capacity was calculated by breakthrough curves at complete exhaustion, which is greater than in the batch experiment. Large concentration gradient occurred continuously at the interface zones as it passes through the column, which leads to high column capacity. While the concentration gradient decreased with time in the batch experiment. Multiple elements column flow experiment revealed the clear sorption preference for Pb^{2+} and Cu^{2+}. Similar results were obtained by Gerente et al. (2004) on biosorption of Pb^{2+}, Cu^{2+} and Ni^{2+} on sugar beet pulp,

Fig. 1 Experimental breakthrough curve for adsorption of metal ions on neem biomaterial
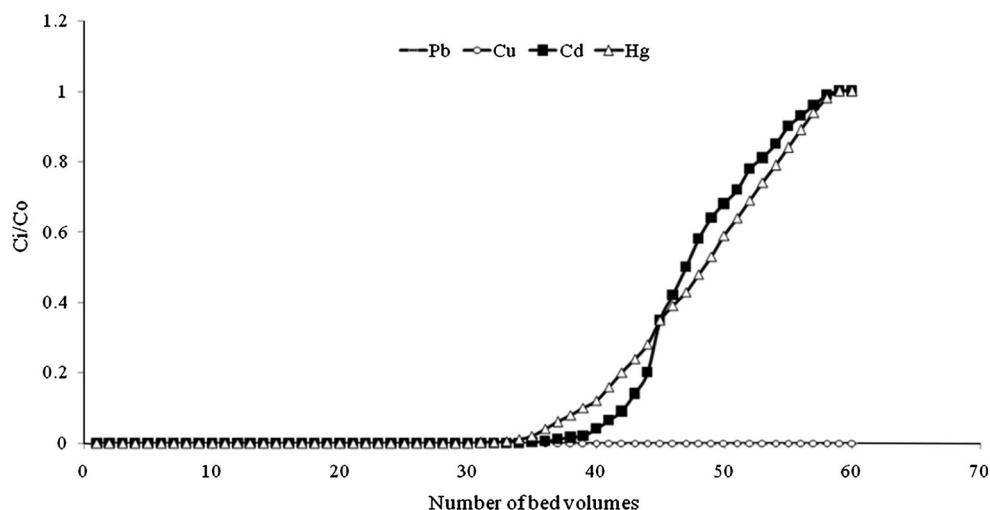
and it was also reported that Ni^{2+} had a less competitive effect.

Figure 2 represents the release of Ca^{2+} and Mg^{2+} with respect to bed volumes. Ca^{2+} ions were consistently released from the biomaterial in a continuously decreasing order up to 60 bed volumes. The presence of ample Ca^{2+} ions in neem biomaterial increased the ability to bind as many as four metals for ion exchange and functional groups which attract and sequester metal ions. In comparison to Ca^{2+} ions, Mg^{2+} ions seemed to be less dependent on Pb^{2+}, Cu^{2+} and Cd^{2+} ions, where as Mg^{2+} ions played a substantial role in an ion exchange of Hg^{2+} ions. Hanzlik et al. (2004) concluded total metal uptake was considerably higher in a mixture than single-ion adsorption.

The FTIR spectrum of biomaterial prepared from neem bark (Fig. 3a) showed several polar groups, –OH, –NH, –C=O, and other amino, sulfonate and phosphonate groups which may be able to interact with metals being currently studied. The biomaterial showed a strong band at 3550–3250 cm^{-1} that refered to the NH and OH vibrational
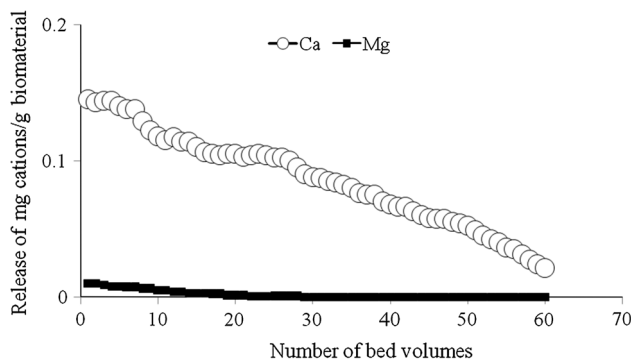

Fig. 2 Release of cations from biomaterial in column adsorption experiment

stretches. A smaller sharp peak at 1180 cm^{-1} may be assigned to the C–N stretches of different amines. A strong absorption band at 1030 cm^{-1} is characteristic of the C–O stretch of alcohols. Two sharp peaks observed at 1690 and 1590 cm^{-1} are indicative of the presence of carbonyl groups associated with amides and carboxyl groups. Peaks around 1505 and 1380 cm^{-1} are assigned to the N–H stretch of primary and secondary amines. The main C–H stretch peak of the carbon polymeric backbone is shown at 2900 cm^{-1}. Broad bands present between 1400 and 1350 cm^{-1} are characteristic of the asymmetric bending vibration of different types of aliphatic carbons and CH_2 bonded to double bonds, aromatics and carbonyls.

Pb loaded biomaterial (Fig. 3b) showed a decrease in the intensity of the hydroxyl and free amine peaks at 3550–3250 cm^{-1}. There is a considerable broadening and shifting of the C–O peak at 1030 cm^{-1} indicating most of the interaction of the bark with Pb occurred through the C–O groups at 1030 cm^{-1} with minor participation of carbonyls.

The FTIR spectrum of Cu loaded biomaterial (Fig. 3c) also showed decreased intensity of the hydroxyl and free amine stretch bands at 3550–3250 cm^{-1} compared to the native bark starting material. Carbonyl peaks for amides and carboxyls showed broadening with bands starting at longer wavelengths. The C–O band for alcohols in the material showed a shift towards longer wavelengths together with a deformation of the main band into different smaller overlapping bands with two maximums at 1050 and 1040 cm^{-1}. The appearance of these additional bands over the area of 1030 cm^{-1} served as evidence the alcohol terminals together with the carbonyls of the bark material are the main functional groups involved in the binding of Cu. The shifting towards longer wavelengths occurring on the Cu loaded material strongly suggested that there is a coordination type binding between these functional groups and the metal.
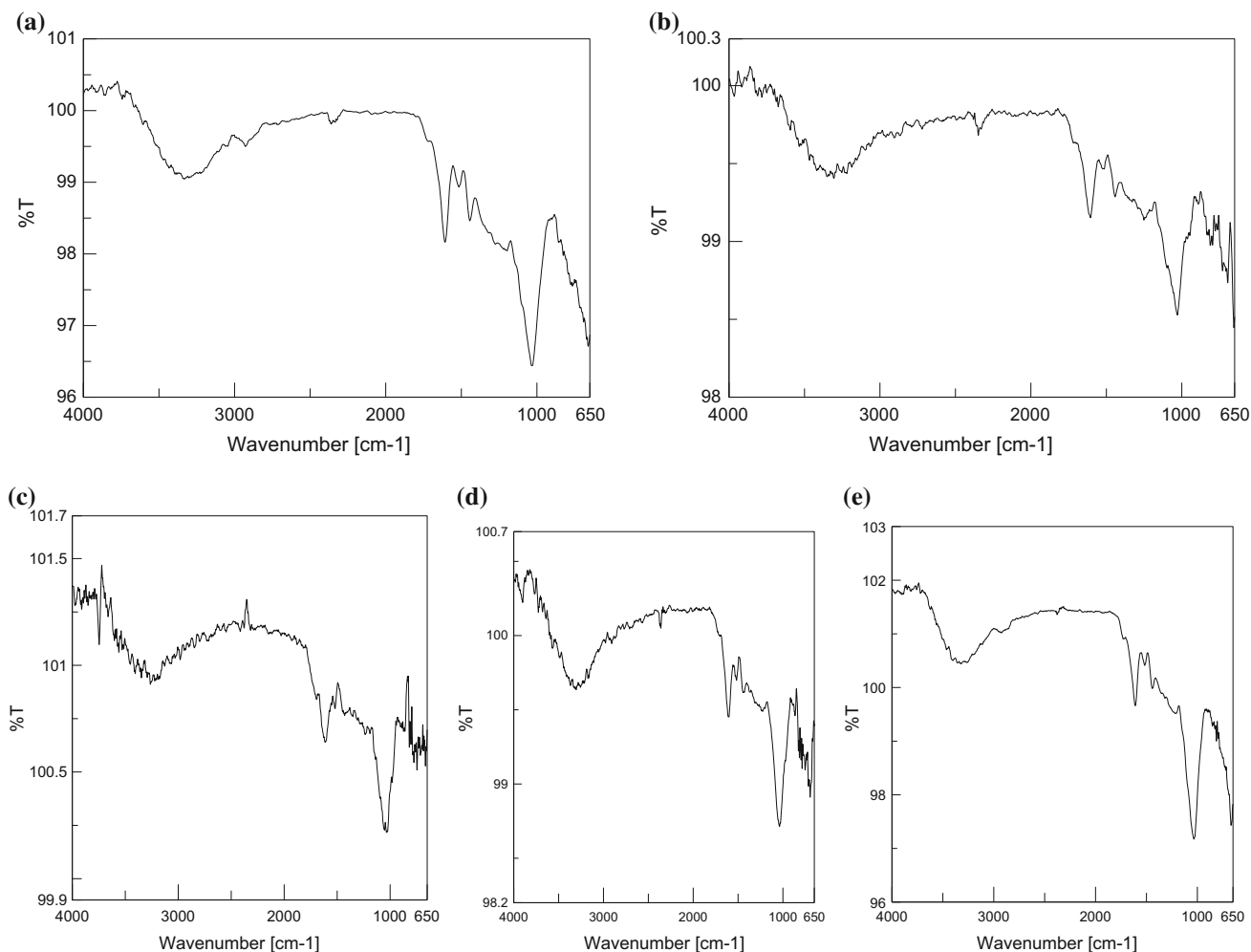
Fig. 3 FTIR of biomaterial and biomaterials loaded with metals. **a** Neem biomaterial (control sample), **b** Pb loaded biomaterial, **c** Cu loaded biomaterial, **d** Cd loaded biomaterial, **e** Hg loaded biomaterial

Cd spectrum (Fig. 3d) showed a shifting and broadening 1690 cm^{-1} band assigned to the amide carbonyl and the appearance of two major overlapping bands at the N–H stretches frequencies of 1505 and 1380 cm^{-1}. The C–O stretch at 1030 cm^{-1} showed the appearance of three overlapping absorptions shifted to longer wavelengths. Changes in the IR absorption described suggest the binding of Cd^{2+} is primarily influenced by the presence of amines and free hydroxyl functional groups in the bark structure. With the exception of Hg, which remained similar to native bark material, all the other metal loaded biomaterial showed significant absorption changes on the fingerprint area which suggested sulfur and phosphates were possibly involved in the binding.

The Hg loaded biomaterial (Fig. 3e) showed a low level of significant changes compared to native bark material, which is indicative of low levels of chemical interactions between the functional groups on the bark surface and Hg. However, the main interaction of Hg could be at the

sulfonate groups ($\sim$1050–1030 cm^{-1}). Peaks below 800 cm^{-1} belong to the fingerprint frequencies with absorptions of the functional groups containing sulfur and phosphates (Munagapati et al. 2010).

Dupont et al. (2006) studied adsorption isotherms of five metal cations (Cr^{3+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) onto five species of barks such as sweet chestnut, oak, Douglas fir, Norway spruce and Scots pine species and concluded Cu^{2+} interacted preferentially with phenolic groups present in lignins and tannins, while Pb^{2+} with carboxylic acid groups in polysaccharides. Similar conclusions have been drawn in the present study. At a higher pH, the percentage of phenolic species becomes relatively more important (Dupont et al. 2003).

Gerente et al. (2006) observed two types of fixation site for Pb^{2+}: the carboxylic sites involved in ion exchange with Ca^{2+} and those taking part in adsorption. The present work described the retention mechanism of metal ions onto the newly developed neem bark based biomaterial.

Whewellite ($C_2CaO_4 \cdot H_2O$) has been identified in the biomaterial, which indicated Ca^{2+} ions are electrovalently bonded with carboxylate ions facilitating the ion exchange mechanism with metal ions. Neem bark can be used as bioindicators, bioaccumulators and biomonitors while determining environmental pressures. This type of structural investigation enabled an accurate prediction of the metal bioaccumulative properties of the material, which in turns helped in providing quantitative information on the metal contamination in the environment around it.

References

Arshad M, Zafar MN, Younis S, Nadeem R (2008) The use of Neem biomass for the biosorption of zinc from aqueous solutions. J Hazard Mater 157(2–3):534–540

Athar M, Farooq U, Hussain B (2007) *Azadirachata indicum* (Neem): an effective biosorbent for the removal of lead (II) from aqueous solutions. Bull Environ Contam Toxicol 79(3):288–292

Bulut Y, Baysal Z (2006) Removal of Pb(II) from wastewater using wheat bran. J Environ Manag 78:107–113

Dupont L, Bouand J, Dumonceau J, Aplincourt M (2003) Metal ions binding onto a lignocellulosic substrate extracted from wheat bran: a NICA–Donnan approach. J Colloid Interface Sci 263:35–41

Dupont MF, Gloaguen V, Guilloton M, Granet R, Krausz P (2006) Study of the chemical interaction between barks and heavy metal cations in the sorption process. J Environ Sci Health A 41(2):149–160

Gerente C, Reddad Z, Abdres Y, Le Cloirec P (2004) Competitive adsorption of metals and organics onto a low cost natural polysaccharide. Environ Technol 25:219–225

Gerente C, Du Mesnil PC, Andres Y, Thibault JF, Le Cloirec P (2006) Removal of metal ions from aqueous solution on low cost natural polysaccharides sorption mechanism approach. React Funct Polym 46:135–144

Gloaguen V, Morvan H (1997) Removal of heavy metal ions from aqueous solution by modified barks. J Environ Sci Health A 32(4):901–912

Hanzlik J, Jehlicka J, Sebek OO, Weishauptova Z, Machovic VV (2004) Multi-component adsorption of Ag(I), Cd(II) and Cu(II) by natural carbonaceous materials. Water Res 38(8):2178–2184

Horsfall M, Abia AA, Spiff AI (2006) Kinetic studies on the adsorption of Cd^{2+}, Cu^{2+} and Zn^{2+} ions from aqueous solutions by cassava (*Manihotsculenta Cranz*) tuber bark waste. Bioresour Technol 97(2):283–291

ICDD (2002) Powder diffraction file release 2002, PDF-2: International Centre for Diffraction Data, Newton Square, PA

Jang A, Seo Y, Bishop PL (2005) The removal of heavy metals in urban runoff by sorption on mulch. Environ Pollut 133(1):117–127

Krishnani KK, Meng X, Boddu VM (2008a) Fixation of heavy metals onto lignocellulosic sorbent prepared from paddy straw. Water Environ Res 80:2165–2174

Krishnani KK, Ayyappan S (2006) Heavy metals remediation of water using plant and lignocellulosic agrowastes. Rev Environ Contam Toxicol 88:64–85

Krishnani KK, Meng X, Christodoulatos C, Boddu VM (2008b) Biosorption mechanism of nine different heavy metals onto biomatrix from rice husk. J Hazard Mater 153:1222–1234

Krishnani KK, Meng X, Dupont L (2009) Metal ions binding onto lignocellulosic biosorbent. J Environ Sci Health A 44:1–12

Maharia RS, Dutta RK, Acharya R, Reddy AV (2010) Heavy metal bioaccumulation in selected medicinal plants collected from Khetri copper mines and comparison with those collected from fertile soil in Haridwar, India. J Environ Sci Health B 45(2):174–181

Masri MS, Reuter FW, Friedman M (1974) Binding of metal cations by natural substances. J Appl Polym Sci 18(3):675–681

MDI (Material's Data Inc) (2005) Jade Version 7.1. California, USA

Munagapati VS, Yarramuthi V, Nadavala SK, Alla SR, Abburi K (2010) Biosorption of Cu(II), Cd(II) and Pd(II) by *Acacia leucophala* bark powder: kinetics, equilibrium and thermodynamics. Chem Eng J 157:357–365

Samecka-Cymerman A, Kosior G, Kempers AJ (2006) Comparison of the moss *Pleurozium schreberi* with needles and bark of *Pinussyl vestris* as biomonitors of pollution by industry in Stalowa Wola (southeast Poland). Ecotoxicol Environ Saf 65(1):108–117

Sekhar CK, Kamala CT, Chary NS, Sastry AR, Rao NT, Vairamani M (2004) Removal of lead from aqueous solutions using an immobilized biomaterial derived from a plant biomass. J Hazard Mater 108:111–117

Xin-Min Z, Zhao X (2003) Mechanism of lead adsorption from aqueous solutions using an adsorbent synthesized from natural condensed tannins. Water Res 37:3905–3912

Xin-Min Z, Miyazaki A, Nakano Y (2001) Mechanisms of lead removal from aqueous solutions using a novel tannin gel adsorbent synthesized from natural condensed tannin. J Chem Eng Jpn 34:1204–1210